Use of *Bacillus velezensis* RI3 and *Pseudomonas psychrophila* P10 strains as plant growth-promoting rhizobacteria in lettuce (*Lactuca sativa* L.)

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ABSTRACT

Lettuce is cultivated on over one million hectares worldwide, with Argentina contributing 40,000 ha. Córdoba province is the second-largest producer in Argentina, but its green belt has seen significant reductions in cultivated areas. New environmentally friendly technologies are needed to improve, sustain and increase lettuce volume production. This study aimed to use plant growth-promoting rhizobacteria (PGPR) as a sustainable alternative to enhance lettuce production (*Lactuca sativa* L.) growth while mitigating environmental impact. Two PGPR strains, *Bacillus velezensis* RI3 and *Pseudomonas psychrophila* P10, were tested in both *in vitro* and field experiments. Parameters such as shoot dry weight, root dry weight, evapotranspiration, and water use efficiency were assessed. The *in vitro* study results demonstrated significant improvements in shoot and root dry weights for both inoculated treatments compared to the control. Field experiments, conducted on 90 m^2 plots, also demonstrated significant increases in shoot dry weight, with *Pseudomonas psychrophila* showing a 28.57 % increase and *Bacillus velezensis* an 8.44 % increase compared to controls. This study highlights the potential of *Bacillus velezensis* and *Pseudomonas psychrophila* as effective biofertilizers, promoting sustainable lettuce cultivation. Further research is recommended to explore the mechanisms of plant-microbe interactions to optimize PGPR application in agricultural practices.

Keywords: bioinputs, horticultural crops, PGPR, crop yield

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RESUMEN

Se cultivan un millón de hectáreas de lechuga a nivel mundial, de las cuales 40 000 están en Argentina. La provincia de Córdoba, el segundo mayor productor del país, ha visto una reducción significativa de su cinturón verde, lo que subraya la necesidad de tecnologías que permitan incrementar la producción y garantizar la sostenibilidad. Este estudio evaluó el uso de rizobacterias promotoras del crecimiento vegetal (PGPR) como alternativa para incrementar la producción de la lechuga (*Lactuca sativa* L.) y reducir el impacto ambiental. Se probaron dos cepas de PGPR, *Bacillus velezensis* RI3 y *Pseudomonas psychrophila* P10, en experimentos *in vitro* y de campo. Se analizaron parámetros como el peso seco de la parte aérea, peso seco radical, evapotranspiración y eficiencia en el uso del agua. Los resultados *in vitro* mostraron mejoras significativas en los pesos secos en ambos tratamientos comparados con el control. En campo, *Pseudomonas psychrophila* mejoró el peso seco aéreo en un 28,57 % y *Bacillus velezensis* en un 8,44 % respecto a los controles. Este estudio subraya el potencial de estas cepas como biofertilizantes, promoviendo una producción de lechuga más sostenible. Más trabajos de investigación son necesarios para optimizar la aplicación de PGPR en la agricultura.

Palabras clave: bioinsumos, cultivos hortícolas, RPVC, aumento de rendimiento

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INTRODUCTION

Lettuce cultivation spans over one million hectares globally. In Argentina, lettuce cultivation extends over 40,000 ha, 28.66 % of the total area of vegetable production (139,585 ha) according to the National Institute of Statistics and Census (Instituto Nacional de Estadística y Censos [INDEC], 2021). The main lettuce cultivation regions are the green belts of Buenos Aires, Mar del Plata, Córdoba, and Santa Fe (Scaturro, 2019). In Córdoba, 86 % of the total vegetable land area (15,803 ha) is cultivated with lettuce 13,590 ha (INDEC, 2021; Viano, 2022). This region represents more than 33 % of total Argentinian lettuce cultivation (Viano, 2022). "Between 2004 and 2019, the vegetable land area decreased by 47 %, and since 1988, it has decreased by 73 %" (Viano, 2022). This reduction has led to increased land rental costs and economic instability (Viano, 2022). Moreover, consumers demand eco-friendly products and more sustainable agricultural practices to produce them. An alternative to mitigate the environmental impact and enhance resource use efficiency is the use of biofertilizers and biostimulants (Khatoon et al., 2020; Nagrale et al., 2023; Pantoja-Guerra et al., 2023; Tabassum et al., 2017).

In the soils, biofertilizers and biostimulants, along with the natural microbial communities, interact with the plant rhizosphere (Bramhachari et al., 2017; Delitte et al., 2021; Ezazi et al., 2021; Ferrarezi et al., 2022; Leveau, 2007). In this region, the application of plant growth-promoting rhizobacteria (PGPR) as biostimulants could promote plant growth and health. The most common PGPR genera include *Azotobacter*, *Pseudomonas*, *Azospirillum*, and *Bacillus* (Kejela et al., 2017; Kour et al., 2019; Kumar et al., 2019; Shah et al., 2021). PGPR has different mechanisms to foster plant growth, classified as either direct or indirect. Direct mechanisms include the production of phytohormones, volatile organic compounds (VOCs), siderophore synthesis, and nutrient solubilization. Indirect mechanisms boost the defense against pathogens and include nutrient competition, antibiotic production, and systemic resistance induction (Kumar et al., 2019; Shah et al., 2021). Vegetable production could thus be promoted throughout the PGPR application (Rai and Nabti, 2017). Several studies indicate that PGPR promote the growth and yield of broccoli by 20 % (Yildirim et al., 2011), lettuce by 10 % (Cipriano et al., 2016), cucumber by > 60 % (Gül et al., 2013), and tomato by > 10 % (Bernabeu et al., 2015), among others.

Considering the bioinputs market trends, *Bacillus* and *Pseudomonas*, after the rhizobia (*e.g., Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, etc.) and *Azospirillum* spp. microorganisms, are two of the most common PGPR genera adopted by farmers. These genera have metabolic versatility that adapts to different soil types and environmental conditions (Baysal et al., 2013; Chauhan et al., 2023; Glick, 2012; Kumar et al., 2019). Recent *Bacillus* and *Pseudomonas* species, such as *B. subtilis*, *B. thuringiensis*, *B. velezensis*, *P. fluorescens*, *P. putida*, and *P. psychrophila*, among others, exhibit PGPR effects (*i.e.*, phytohormone production, nutrient uptake and solubilization, alleviation of biotic and abiotic stress) and are widely employed in different crop systems (Nguyen et al., 2019; Rabbee et al., 2019; Singh et al., 2022; Win et al., 2018). These species have been shown to increase yields in different crops by over 5 %, with an average increase of 20 % (wheat, chickpea, soybeans, corn, and bell pepper) (Dey et al., 2004; Laranjeira et al., 2022; Mosela et al., 2022; Steiner et al., 2021; Stoll et al., 2021; Turan et al., 2012). Recent studies have shown that *B. velezensis* RI3 and *P. psychrophila* P10 have increased the yield in peanuts from 8 % to 33 % (Bigatton, Ayoub et al., 2024; Bigatton, Verdenelli et al., 2024). These microorganisms have also shown significant improvements in chickpeas, corn, sorghum, rye, wheat, and soybeans, following similar trends to those achieved in peanuts (unpublished results). Considering these results and the necessity of generating information for the horticultural sector, this study aimed to assess the biostimulant and growth-promoting effects of *B. velezensis* RI3 and *P. psychrophila* P10 on lettuce (*Lactuca sativa* L.) cultivation. In this study, native *B. velezensis* RI3 and *P. psychrophila* P10 were applied to lettuce cultivation in both pot and field trials to evaluate the effects of these PGPR on lettuce growth and yield.

MATERIALS AND METHODS

Two experiments were conducted: (i) an *in vitro* pot experiment and (ii) a field experiment. The *in vitro* experiment (i) was carried out at the Agricultural Microbiology Department of the Faculty of Agricultural Sciences, National University of Córdoba (FCA-UNC), Argentina, in a growth chamber with controlled conditions of 28 °C temperature and a 14 h light/10 h dark photoperiod. Two PGPR strains were evaluated, *Bacillus velezensis* RI3 and *Pseudomonas psychrophila* P10, previously isolated and genetically characterized by the Agricultural Microbiology Laboratory of FCA-UNC (accession number MT377597 and MT377608, GeneBank) (Clark et al., 2016). The strains were cultured and incubated in tryptic soy broth at 28-30 °C with shaking (150 rpm) for 24 h until they reached 1 x 10^9 Colony Forming Units (CFU) mL^{-1}. A lettuce cultivar (*Lactuca sativa* L.) var. Elisa, commonly referred to as "mantecosa" was used. Before sowing, seeds were disinfected and treated with Thiabendazole (15 g L^{-1}), Fludioxonil (2.5 g L^{-1}), and Metalaxyl-M (2 g L^{-1}), and inoculated at a concentration of 1 x 10^6 CFU $seed^{-1}$. Control treatment seeds were treated with sterile water in the same proportion as the inoculant. Each treatment comprised seven pots of 3 L capacity. The substrate was 1:1 (v/v) mixture of sterilized soil (Typic Haplustoll) and sand. Pots were arranged in a completely randomized block design.

At the beginning of the experiment, pots were irrigated to 100 % field capacity, determined by gravimetric method. After the lettuce emergency, all pots were maintained at 60 % field capacity with daily watering. Daily evapotranspiration of each pot was computed. The experiment concluded forty days after sowing. The parameters evaluated were: Chlorophyll Content Index (CCI), root dry weight (RDW, in g $plant^{-1}$), shoot dry weight (SDW, in g $plant^{-1}$), total dry weight (TDW, in g $plant^{-1}$), leaf weight ratio (LWR), evapotranspiration expressed as plant water consumption (PWC, in mL $plant^{-1}$), and Water Use Efficiency (WUE) expressed as TDW (in g $plant^{-1}$) / PWC (in mL $plant^{-1}$).

The field experiment was conducted in the green belt of Córdoba City, Argentina (31° 21' 4.96'' S, 64° 5' 33.05'' W) (Figure 1). Prior to field transplanting, lettuce seedlings were sown in seedbeds using the same strains as the *in vitro* experiment. Seedlings with three or four leaves were transplanted to the field. In the field, each plot had 90 m^2, five rows (0.5 m between each row) accounting for 3 m wide and 30 m long, per replicate. Each treatment had three replicates. For biomass production, thirty plants per replicate were harvested. Drip irrigation was used, and all the plots were maintained free of weeds, pests, and diseases. When plants reached marketable size, ninety days after sowing, they were harvested. The parameters evaluated were shoot dry weight (SDW, in g $plant^{-1}$) and shoot fresh weight (SFW, in g $plant^{-1}$).

Data were processed and analyzed using the InfoStat® statistical software (Di-Rienzo et al., 2017). A Generalized Linear Model (GLM) and Fisher's LSD mean comparison test ($p < 0.05$) were performed.

RESULTS

Considering the SDW ($p < 0.0002$) and TDW ($p < 0.0001$), PGPR treatments differed significantly from the control (Table 1). No significant differences were observed between PGPR treatments. Considering SDW, PGPR (P10 > RI3) were 44 % higher than control (Table 1). The TDW was 0.77 g $plant^{-1}$ for RI3 and 0.71 g $plant^{-1}$ for P10, 97 % and 82 % higher than control, respectively (Table 1).

For RDW ($p < 0.0001$), the RI3 showed 0.46 g $plant^{-1}$, followed by P10 with 0.39 g $plant^{-1}$, 170 %, and 110 % over the control, respectively (Table 1, Figure 5). Considering the field experiment (Figure 1), P10 showed the highest SDW, 19.8 g $plant^{-1}$, 28.5 % over the control (15.4 g $plant^{-1}$). RI3 followed with 16.7 g $plant^{-1}$, 8.44 %) above the control (Table 2).

The chlorophyll content index (CCI) (Table 3) revealed no significant disparities among the treatments in both experiments.

Daily evapotranspiration ($p < 0.0001$) (Figure 2) exhibited a gradual decrease in water consumption over time in the *in vitro* experiment. This decline

Figure 1. Field experiment, every plot consisting of five rows. The experiment consists of a total of nine plots.

Table 2. Determination of lettuce crop yield in the field trial, under the influence of PGPR bacterial inoculation. The data shown is the average values of the measured parameters.

Treatment	SFW (g)		SDW(g)	
P10	$346.7^{\pm 24.7}$	**a**	$19.8^{\pm 0.49}$	**a**
RI3	$293.3^{\pm 37.6}$	**b**	$16.7^{\pm 0.52}$	**b**
Control	$267.0^{\pm 19.5}$	**b**	$15.4^{\pm 0.66}$	**c**

SFW: shoot fresh weight/plant; SDW: shoot dry weight/plant. P10: *P. psychrophila;* RI3: *B. velezensis*. Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

Table 1. Determination of lettuce crop SDW, RDW, TDW and LWR in a growth chamber, under the influence of PGPR bacterial inoculation. The data shown is the average values of the measured parameters.

Treatment	SDW (g)		RDW (g)		TDW(g)		LWR	
RI3	$0.321^{\pm 0.016}$	**a**	$0.456^{\pm 0.015}$	**a**	$0.777^{\pm 0.025}$	**a**	$41.16^{\pm 1.93}$	**a**
P10	$0.324^{\pm 0.017}$	**a**	$0.386^{\pm 0.016}$	**b**	$0.711^{\pm 0.028}$	**a**	$45.07^{\pm 2.11}$	**ab**
Control	$0.223^{\pm 0.017}$	**b**	$0.169^{\pm 0.016}$	**c**	$0.392^{\pm 0.028}$	**b**	$50.11^{\pm 2.11}$	**b**

SDW: shoot dry weight; RDW: root dry weight; TDW: total dry weight; LWR: leaf weight ratio; P10: *P. psychrophila*; RI3: *B. velezensis*. Means denoted by a different letter indicate significant differences between treatments (LSD; $p < 0.05$).

Table 3. Determination of Chlorophyll Content Index (CCI) in lettuce plants under the influence of PGPR bacterial inoculation. The data shown is the average values of the measured parameter.

Treatment	*CCI	
Control	$4.77^{\pm 0.27}$	**a**
RI3	$4.83^{\pm 0.27}$	**a**
P10	$5.00^{\pm 0.27}$	**a**

CCI: Chlorophyll Content Index. RI3: *B. velezensis*; P10: *P. psychrophile*. Means denoted by a different letter indicate significant differences between treatments (p < 0.05).

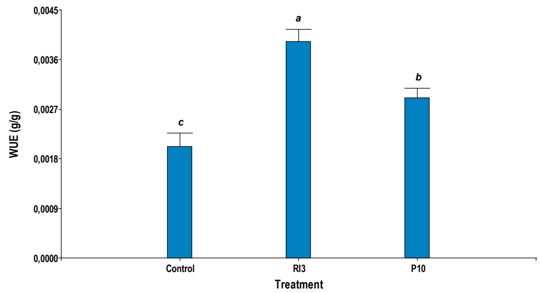

Figure 3. Water use efficiency expressed in grams of total dry matter per gram of water consumed, in lettuce crop in a growth chamber under the effect of PGPR bacterial inoculation. Different letters indicate significant differences (LSD test; p ≤ 0.05).

can be attributed to the initial irrigation at 100 % field capacity, which was then maintained at 60 % throughout the experiment. P10 after emergency, maintained high evapotranspiration rates (39.91 mL plant^{-1}) compared to RI3 (31.74 mL plant^{-1}) and control rates (33.22 mL plant^{-1}).

Nonetheless, in terms of Water Use Efficiency (WUE) ($p < 0.0001$), RI3 exhibited the highest value at 0.00393 g mL^{-1}, compared to P10 with 0.00291 g mL^{-1} (Figure 3). These WUE were 94 % and 44 % higher than the control treatment (0.00202 g mL^{-1}).

Finally, regarding the plant water consumption, RI3 showed the lowest water consumption with 153.33 mL plant^{-1} (Figure 4) and statistically differed from P10 and control (216.98 mL plant^{-1} and 195.08 mL plant^{-1}).

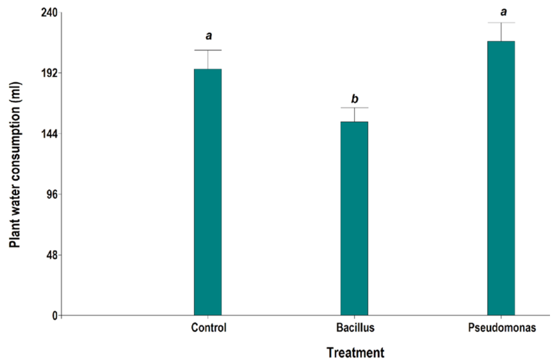

Figure 4. Plant water consumption in milliliters, in lettuce crop in a growth chamber under the effect of PGPR bacterial inoculation. Different letters indicate significant differences (LSD test; p ≤ 0.05).

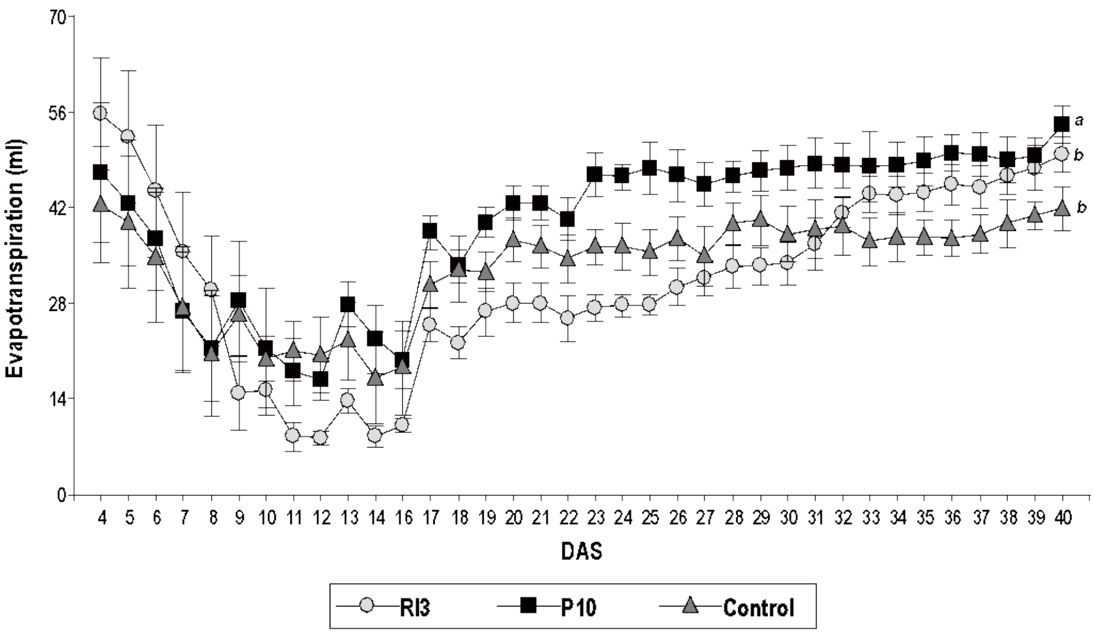

Figure 2. Average evapotranspiration in milliliters per day for each treatment. The data presented indicate the mean of the measured parameters. Different letters indicate significant differences (LSD test; p ≤ 0.05). DAS: Days after sowing.

Figure 5. Comparison between the average size of lettuce seedlings in the control treatment versus those inoculated with *Bacillus* strain and *Pseudomonas* strain

DISCUSSION

This work addressed the effects of PGPR on lettuce cultivation with *in vitro* and field approaches conducted at the same time, which other reports overlooked because they mostly studied the PGPR effects under controlled conditions without field validation of the same strains. This research revealed that PGPR strains enhanced shoot dry weight and root dry weight under both *in vitro* and field conditions. Additionally, PGPR improved plant water content and water use efficiency in the *in vitro* experiment. These increases could be attributed to the PGPR direct mechanisms, such as phytohormone production (Gouda et al., 2018). As demonstrated by Bigatton, Ayoub et al. (2024), these PGPR strains increase hormone levels (*e.g.,* auxins, gibberellins, and cytokinin) in the peanut plant tissues. These growth-promoting mechanisms lead to enhanced root development, which increases the capacity of the plant to absorb water and nutrients. This results in greater shoot dry weight, root dry weight, and also water use efficiency (Kour et al., 2020; Sarkar et al., 2022; Shah et al., 2021). Furthermore, PGPR increase the availability of immobile nutrients like phosphorus by releasing organic acids and acid phosphatases, facilitating nutrient solubilization (Zarei et al., 2019).

Different research on PGPR in lettuce showed biomass increasing after PGPR inoculation. Acurio Vásconez et al. (2020) showed a 283 % increase in root dry matter when lettuce was inoculated with *Bacillus megaterium* (Acurio Vásconez et al., 2020). Gagliano et al. (2016) registered a 7.7 % increase in shoot weight in lettuce plants inoculated with *Bacillus subtilis* compared to the non-inoculated control. Khosravi et al. (2018) reported an 8 % increase in shoot dry matter compared to the control in a greenhouse experiment at Shiraz University in Iran using *Pseudomonas fluorescens* as an inoculant. Cipriano et al. (2016) observed an 11-14 % increase in shoot dry matter in lettuce inoculated with different *Pseudomonas* spp. strains. These results were consistent with these experiments, which showed an increase of over 80 % in shoot dry weight and root dry weight.

Considering the water use efficiency, these results in the P10 treatment (0.00393 g mL^{-1}) were coincident with Fernández et al. (2023), who demonstrated that lettuce water use efficiency ranged from 0.00021 g mL^{-1} to 0.00293 g mL^{-1} in thirteen trials. Another greenhouse trial in Argentina, evaluating the Elisa variety (used in this study), reported a water use efficiency of 0.0026 g mL^{-1} during the initial vegetative stage (Fiorilo, 2018), this value was similar to the control treatment of the present study. A separate greenhouse study in Argentina using the “mantecosa” lettuce variety showed water use efficiency values ranging from 0.00112 g mL^{-1} to 0.00236 g mL^{-1} (Defilipis et al., 2006). Slightly lower values were reported in an Indonesian study, with water use efficiency ranging from 0.00104 g mL^{-1} to 0.00163 g mL^{-1} (Endah Diansari et al., 2019), and even lower values were found in Ecuador, ranging from 0.00012 g mL^{-1} to 0.00008 g mL^{-1} (Rivera Beltrán, 2020). Conversely, Michelon et al. (2020) evaluated a Simplified Soilless Cultivation system across four experiments using five lettuce cultivars, reporting water use efficiency values from 0.00854 g mL^{-1} to 0.0384 g mL^{-1}, significantly higher than those in this study. Similarly, Nederhoff and Stanghellini (2010) reported a water use efficiency value of 0.0075 g mL^{-}1 for lettuce, based on an average from 65 countries.

CONCLUSIONS

In conclusion, inoculation with *Bacillus velezensis* RI3 and *Pseudomonas psychrophila* 10 significantly improved lettuce growth, with notable increases in shoot and root weights compared to the control. These findings highlight the potential of these PGPR native strains as biostimulants for enhancing lettuce production. Future research should focus on elucidating the underlying mechanisms of plant-microbe interactions to optimize their application in agricultural practices.

REFERENCES

Acurio Vásconez, R. D., Mamarandi Mossot, J. E., Ojeda

Shagñay, A. G., Tenorio, E. M., Chiluisa Utreras, V. P., and Vaca Suquillo, I. de los Á. (2020). Evaluación de Bacillus spp. Como rizobacterias promotoras del crecimiento vegetal (RPCV) en brócoli (*Brassica oleracea* var. Italica) y lechuga (*Lactuca sativa*). *Ciencia & Tecnología Agropecuaria, 21*(3), 1–16. https://doi.org/10.21930/rcta.vol21_num3_art:1465

Baysal, Ö., Lai, D., Xu, H., Siragusa, M., Çalı kan, M., Carimi, F., Teixeira da Silva, J. A., and Tör, M. (2013). A Proteomic Approach Provides New Insights into the Control of Soil-Borne Plant Pathogens by *Bacillus* Species. *PLoS ONE, 8*(1), e53182. https://doi.org/10.1371/journal.pone.0053182

Bernabeu, P. R., Pistorio, M., Torres Tejerizo, G. A., Estrada De los Santos, P., Galar, M. L., Boiardi, J. L., and Luna, M. F. (2015). Colonization and plant growth-promotion of tomato by Burkholderia tropica. *Scientia Horticulturae, 191*, 113-120. https://doi.org/10.1016/j.scienta.2015.05.014

Bigatton, E. D., Ayoub, I., Palmero, F., Castillejo, M. Á., Vázquez, C., Lucini, E. I., and Haro, R. J. (2024). Plant-growth promoting rhizobacteria on peanuts: Effects on yield determination, growth rates, and radiation use efficiency in field trials in Argentina. *European Journal of Agronomy*, *154*, 127113. https://doi.org/10.1016/j.eja.2024.127113

Bigatton, E. D., Verdenelli, R. A., Haro, R. J., Ayoub, I., Barbero, F. M., Martín, M. P., Dubini, L. E., Jorrín Novo, J. V., Lucini, E. I., and Castillejo, M. Á. (2024). Metagenomic Analysis to Assess the Impact of Plant Growth-Promoting Rhizobacteria on Peanut (*Arachis hypogaea* L.) Crop Production and Soil Enzymes and Microbial Diversity. *Journal of Agricultural and Food Chemistry, 72*(40), 22385–22397. https://doi.org/10.1021/acs.jafc.4c05687

Bramhachari, P. V., Nagaraju, G. P., and Kariali, E. (2017). Metagenomic Approaches in Understanding the Mechanism and Function of PGPRs: Perspectives for Sustainable Agriculture. In V. S. Meena, P. K. Mishra, J. K. Bisht, & A. Pattanayak (Eds.), *Agriculturally Important Microbes for Sustainable Agriculture* (163–182). Springer. https://doi.org/10.1007/978-981-10-5589-8_8

Chauhan, M., Kimothi, A., Sharma, A., and Pandey, A. (2023). Cold adapted Pseudomonas: Ecology to Biotechnology. *Frontiers in Microbiology*, *14*, 1218708. https://doi.org/10.3389/fmicb.2023.1218708

Cipriano, M. A. P., Lupatini, M., Lopes-Santos, L., Da Silva, M. J., Roesch, L. F. W., Destéfano, S. A. L., Freitas, S. S., and Kuramae, E. E. (2016). Lettuce and rhizosphere microbiome responses to growth promoting Pseudomonas species under field conditions. *FEMS Microbiology Ecology, 92*(12), 197. https://doi.org/10.1093/femsec/fiw197

Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., and Sayers, E. W. (2016). GenBank. *Nucleic Acids Research, 44*(D1), D67–D72. https://doi.org/10.1093/nar/gkv1276

Defilipis, C., Pariani, S., Jimenez, A., and Bouzo, C. (2006). *Respuesta al riego de lechuga (Lactuca sativa L.) cultivada en invernadero.* En III Jornadas de Actualización en Riego y Fertirriego. Facultad de Ciencias Agrarias, Mendoza, Argentina.

Delitte, M., Caulier, S., Bragard, C., and Desoignies, N. (2021). Plant Microbiota Beyond Farming Practices: A Review. *Frontiers in Sustainable Food Systems, 5*, 624203. https://doi.org/10.3389/fsufs.2021.624203

Dey, R., Pal, K. K., Bhatt, D. M., and Chauhan, S. M. (2004). Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. *Microbiological Research, 159*(4), 371–394. https://doi.org/10.1016/j.micres.2004.08.004

Di-Rienzo, J. A., Casanoves, F., Balzarini, M. G., González, L., Tablada, M., and Robledo, W. (2017). InfoStat versión 2017. Grupo InfoStat, FCA, Universidad Nacional de Córdoba.

Endah Diansari, L., Saptomo, S., and Setiawan, B. I. (2019). Water and Land Productivity of Lettuce *(Lactuca sativa*) Cultivation on Floating Pot in Wetland. *Sriwijaya Journal of Environment, 4*(2), 104–108. https://doi.org/10.22135/sje.2019.4.2.104-108

Ezazi, R., Ahmadzadeh, M., Majidian, S., Stefani, E., Pindo, M., and Donati, C. (2021). Responses of cucumber (*Cucumis sativus* L.) rhizosphere microbial community to some agronomic management practices. *FEMS Microbiology Ecology, 97*(8), 107. https://doi.org/10.1093/femsec/fiab107

Ferrarezi, J. A., Carvalho-Estrada, P. D. A., Batista, B. D., Aniceto, R. M., Tschoeke, B. A. P., Andrade, P. A. D. M., Lopes, B. D. M., Bonatelli, M. L., Odisi, E. J., Azevedo, J. L., and Quecine, M. C. (2022). Effects of inoculation with plant growth-promoting rhizobacteria from the Brazilian Amazon on the bacterial community associated with maize in field. *Applied Soil Ecology, 170*, 104297. https://doi.org/10.1016/j.apsoil.2021.104297

Fernández, H. A., Salazar-Moreno, R., Fitz-Rodríguez, E., López Cruz, I. L., Schmidt, U., and Dannehl, D. (2023). Rendimientos y eficiencia en el uso del agua de lechuga y tomate cherry en jardines urbanos. *Tecnología y Ciencias del Agua, 14*(5), 220–256. https://doi.org/10.24850/j-tyca-14-05-05

Fiorilo, M. J. (2018). *Consumo de agua y crecimiento de tres variedades de lechuga (Lactuca sativa L.) en etapa vegetativa inicial* [Undergraduate Thesis]. Universidad Nacional de Luján. https://ri.unlu.edu.ar/xmlui/handle/rediunlu/1144

Gagliano, E., Castresana, J. E., and Díaz, B. M. (2016). Efectos de aplicación de mezcla de *Trichoderma harzianum* y *viridae, Bacillus subtillis* en lechuga sobre el rendimiento comercial. *Horticultura Argentina,* 35(88), 63. Resúmenes de Horticultura - XXXVIII Congreso Argentino de Horticultura. https://www.horticulturaar.com.ar/es/publicacion/88/

Glick, B. R. (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications. *Scientifica, 5*, 963401.

Gouda, S., Kerry, R. G., Das, G., Paramithiotis, S., Shin, H. S., and Patra, J. K. (2018). Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiological Research, 206*, 131–140. https://doi.org/10.1016/j.micres.2017.08.016

Gül, A., Özaktan, H., Kıdo lu, F., and Tüzel, Y. (2013). Rhizobacteria promoted yield of cucumber plants grown in perlite under Fusarium wilt stress. *Scientia Horticulturae, 153*, 22–25. https://doi.org/10.1016/j.scienta.2013.01.004

Kejela, T., Thakkar, V. R., and Patel, R. R. (2017). A novel strain of Pseudomonas inhibits Colletotrichum gloeosporioides and Fusarium oxysporum infections and promotes germination of coffee. *Rhizosphere, 4*, 9–15. https://doi.org/10.1016/j.rhisph.2017.05.002

Khatoon, Z., Huang, S., Rafique, M., Fakhar, A., Kamran, M. A., and Santoyo, G. (2020). Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems. *Journal of Environmental Management, 273*, 111118. https://doi.org/10.1016/j.jenvman.2020.111118

Khosravi, A., Zarei, M., and Ronaghi, A. (2018). Effect of PGPR, Phosphate sources and vermicompost on growth and nutrients uptake by lettuce in a calcareous soil. *Journal of Plant Nutrition*, *41*(1), 80–89. https://doi.org/10.1080/01904167.2017.1381727

Kour, D., Rana, K. L., Yadav, A. N., Yadav, N., Kumar, M., Kumar, V., Vyas, P., Dhaliwal, H. S., and Saxena, A. K. (2020). Microbial biofertilizers: Bioresources and eco-friendly technologies for agricultural and environmental sustainability. *Biocatalysis and Agricultural Biotechnology, 23*, 101487. https://doi.org/10.1016/j.bcab.2019.101487

Kour, D., Rana, K. L., Yadav, N., Yadav, A. N., Kumar, A., Meena, V. S., Singh, B., Chauhan, V. S., Dhaliwal, H. S., and Saxena, A. K. (2019). Rhizospheric Microbiomes: Biodiversity, Mechanisms of Plant Growth Promotion, and Biotechnological Applications for Sustainable Agriculture. In A. Kumar and V. S. Meena, *Plant Growth Promoting Rhizobacteria for Agricultural Sustainability* (19–65). Springer. https://doi.org/10.1007/978-981-13-7553-8_2

Kumar, A., Patel, J. S., Meena, V. S., and Srivastava, R. (2019). Recent advances of PGPR based approaches for stress tolerance in plants for sustainable agriculture. *Biocatalysis and Agricultural Biotechnology, 20*(4), 101271. https://doi.org/10.1016/j.bcab.2019.101271

Laranjeira, S., Reis, S., Torcato, C., Raimundo, F., Ferreira, L., Carnide, V., Fernandes-Silva, A., and Marques, G. (2022). Use of Plant-Growth Promoting Rhizobacteria and Mycorrhizal Fungi Consortium as a Strategy to Improve Chickpea (*Cicer arietinum* L.) Productivity under Different Irrigation Regimes. *Agronomy, 12*(6), 1383. https://doi.org/10.3390/agronomy12061383

Instituto Nacional de Estadística y Censos (INDEC). (2021). *Censo Nacional Agropecuario 2018. Resultados definitivos.* https://www.indec.gob.ar/ftp/cuadros/economia/cna2018_resultados_definitivos.pdf

Leveau, J. H. J. (2007). The magic and menace of metagenomics: Prospects for the study of plant growth-promoting rhizobacteria. *European Journal of Plant Pathology, 119*(3), 279–300. https://doi.org/10.1007/s10658-007-9186-9

Michelon, N., Pennisi, G., Myint, N. O., Dall'Olio, G., Batista, L. P., Salviano, A. A. C., Gruda, N. S., Orsini, F., and Gianquinto, G. (2020). Strategies for Improved Yield and Water Use Efficiency of Lettuce (*Lactuca sativa* L.) through Simplified Soilless Cultivation under Semi-Arid Climate. *Agronomy, 10*(9), 1379. https://doi.org/10.3390/agronomy10091379

Mosela, M., Andrade, G., Massucato, L. R., De Araújo Almeida, S. R., Nogueira, A. F., De Lima Filho, R. B., Zeffa, D. M., Mian, S., Higashi, A. Y., Shimizu, G. D., Teixeira, G. M., Branco, K. S., Ventura Faria, M., Giacomin, R. M., Scapim, C. A., and Gonçalves, L. S. A. (2022). *Bacillus velezensis* strain Ag75 as a new multifunctional agent for biocontrol, phosphate solubilization and growth promotion in maize and soybean crops. *Scientific Reports, 12*(1), 15284. https://doi.org/10.1038/s41598-022-19515-8

Nagrale, D. T., Chaurasia, A., Kumar, S., Gawande, S. P., Hiremani, N. S., Shankar, R., Gokte-Narkhedkar, N., Renu, K. and Prasad, Y. G. (2023). PGPR: The treasure of multifarious beneficial microorganisms for nutrient mobilization, pest biocontrol and plant growth promotion in field crops. *World Journal of Microbiology and Biotechnology, 39*(4), 100. https://doi.org/10.1007/s11274-023-03536-0

Nederhoff, E., and Stanghellini, C. (2010). Water use efficiency of tomatoes in greenhouses and hydroponics. *Practical Hydroponics & Greenhouses*, 52–59.

Nguyen, M. L., Glaes, J., Spaepen, S., Bodson, B., Du Jardin, P., and Delaplace, P. (2019). Biostimulant effects of Bacillus strains on wheat from in vitro towards field conditions are modulated by nitrogen supply. *Journal of Plant Nutrition and Soil Science, 182*(3), 325–334. https://doi.org/10.1002/jpln.201700610

Pantoja-Guerra, M., Valero-Valero, N., and Ramírez, C. A. (2023). Total auxin level in the soil–plant system as a modulating factor for the effectiveness of PGPR inocula: A review. *Chemical and Biological Technologies in Agriculture, 10*(1), 6. https://doi.org/10.1186/s40538-022-00370-8

Rabbee, M., Ali, Md., Choi, J., Hwang, B., Jeong, S., and Baek, K. (2019). *Bacillus velezensis*: A

Valuable Member of Bioactive Molecules within Plant Microbiomes. *Molecules, 24*(6), 1046. https://doi.org/10.3390/molecules24061046

Rai, A. and Nabti, E. (2017). Plant Growth-Promoting Bacteria: Importance in Vegetable Production. In A. Zaidi and M. S. Khan (Eds.), *Microbial Strategies for Vegetable Production* (23–48). Springer. https://doi.org/10.1007/978-3-319-54401-4_2

Rivera Beltrán, J. F. (2020). *Evaluación del comportamiento del cultivo de lechuga (Lactuca sativa L.) y eficiencia del uso de agua utilizando poliacrilato de potasio en la Granja Experimental La Pradera, Imbabura.* [Undergraduate Thesis]. Universidad Técnica del Norte.

Sarkar, B., Kumar, C., Pasari, S., Goswami, B., and Kumar Koshariya, A. (2022). Review On Pseudomonas Fluorescens: A Plant Growth Promoting Rhizobacteria. *Journal of Positive School Psychology, 6*, 2701–2709.

Scaturro, G. N. (2019). *Evaluación de dos sistemas de producción de lechuga en hidroponia y un cultivo tradicional bajo cubierta* [Undergraduate Thesis]. Universidad Nacional de Luján.

Shah, A., Nazari, M., Antar, M., Msimbira, L. A., Naamala, J., Lyu, D., Rabileh, M., Zajonc, J., and Smith, D. L. (2021). PGPR in Agriculture: A Sustainable Approach to Increasing Climate Change Resilience. *Frontiers in Sustainable Food Systems, 5*. https://doi.org/10.3389/fsufs.2021.667546

Singh, P., Singh, R. K., Zhou, Y., Wang, J., Jiang, Y., Shen, N., Wang, Y., Yang, L., and Jiang, M. (2022). Unlocking the strength of plant growth promoting *Pseudomonas* in improving crop productivity in normal and challenging environments: A review. *Journal of Plant Interactions, 17*(1), 220–238. https://doi.org/10.1080/17429145.2022.2029963

Steiner, F., De Queiroz, L. F. M., Zuffo, A. M., Da Silva, K. C., and De Oliveira Lima, I. M. (2021). Peanut response to co-inoculation of *Bradyrhizobium* spp. and *Azospirillum brasilense* and molybdenum application in sandy soil of the Brazilian Cerrado. *Agronomy Journal, 113*(1), 623–632. https://doi.org/10.1002/agj2.20519

Stoll, A., Salvatierra-Martínez, R., González, M., Cisternas, J., Rodriguez, Á., Vega-Gálvez, A., and Bravo, J. (2021). Importance of crop phenological stages for the efficient use of PGPR inoculants. *Scientific Reports, 11*(1), 19548. https://doi.org/10.1038/s41598-021-98914-9

Tabassum, B., Khan, A., Tariq, M., Ramzan, M., Iqbal Khan, M. S., Shahid, N., and Aaliya, K. (2017). Bottlenecks in commercialisation and future prospects of PGPR. *Applied Soil Ecology, 121*, 102–117. https://doi.org/10.1016/j.apsoil.2017.09.030

Turan, M., Gulluce, M., Von Wirén, N., and Sahin, F. (2012). Yield promotion and phosphorus solubilization by plant growth–promoting rhizobacteria in extensive wheat production in Turkey. *Journal of Plant Nutrition and Soil Science, 175*(6), 818–826. https://doi.org/10.1002/jpln.201200054

Viano, L. (2022, March 21). Cinturón verde: La falta de tierra impacta en la producción de verduras de Córdoba. *La Voz del Interior*.

Win, K., Oo, A., Ohkama-Ohtsu, N., and Yokoyama, T. (2018). *Bacillus Pumilus* Strain TUAT-1 and Nitrogen Application in Nursery Phase Promote Growth of Rice Plants under Field Conditions. *Agronomy, 8*(10), 216. https://doi.org/10.3390/agronomy8100216

Yildirim, E., Karlidag, H., Turan, M., Dursun, A., and Goktepe, F. (2011). Growth, Nutrient Uptake, and Yield Promotion of Broccoli by Plant Growth Promoting Rhizobacteria with Manure. *HortScience, 46*(6), 932–936. https://doi.org/10.21273/HORTSCI.46.6.932

Zarei, T., Moradi, A., Kazemeini, S. A., Farajee, H., and Yadavi, A. (2019). Improving sweet corn (*Zea mays* L. var saccharata) growth and yield using *Pseudomonas fluorescens* inoculation under varied watering regimes. *Agricultural Water Management, 226*, 105757. https://doi.org/10.1016/j.agwat.2019.105757